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The ability of antimicrobial peptides (AMPs) to target and lyse the harmful microbial membrane over that of a host's is a unique characteristic, making these innate immune effectors promising candidates to fill a growing therapeutic void resulting from antibiotic drug resistance. This selectivity is believed to depend on the chemical and structural properties of the lipids that comprise the cell membrane. The selectivity of AMPs can be based on the electrostatic attraction of these predominately cationic peptides for the bacterial membrane surface heavily populated with negatively charged lipid components. We have previously shown with atomic force microscopy that zwitterionic dimyristoylphosphatidylcholine (DMPC) bilayers display concentration-dependent structural transformations induced by protegrin-1 (PG-1) that progress from finger-like instabilities at bilayer edges, to the formation of pores, and finally to a network of worm-like micelles. The increasing degree of membrane disruption in charge-neutral membranes demonstrates that a more complex interaction than that suggested by a simple electrostatic argument is needed to explain AMP selectivity. We propose that in addition to an electrostatic element, specific membrane compositional differences between host and pathogen tunes AMP activity to selectively disrupt microbial membranes. We have tailored our investigations to utilize membrane components which eukaryotes and prokaryotes contain in drastically different proportions, specifically the presence and absence of cholesterol. In these results we have employed a variety of biophysical techniques to elucidate how increasing cholesterol content in both phospholipid monolayers and bilayers attenuates the ability of PG-1 to induce membrane disruption. Atomic force microscopy and isothermal titration calorimetry were used to assess the propensity for peptide insertion and pore formation. X-ray and neutron reflectivity measurements were advantageous in providing molecular level detail on the location and orientation of PG-1 with respect to the membrane.

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What Vesicle Leakage Reveals about Antimicrobial Activity (and What It Doesn't)

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The mode of action of antimicrobial peptides and their mimics is often assessed by vesicle leakage experiments, and most of these compounds are believed to act by membrane permeabilization. This work aims at improving the interpretation of vesicle leakage data in general, and at understanding and optimizing the fungicidal activity of nylon-3 polymers. We have studied the membrane permeabilizing properties of the cationic homopolymer poly-NM (Liu, R et al. JACS, 2013, 135, 5270), which displays significant antifungal activity, and two related cationic/hydrophobic binary copolymers using the lifetime-based leakage assay of calcein-loaded vesicles. We compared the results with biological activities against Candida albicans. Poly-NM induces all-or-none leakage of vesicles that are made from yeast polar lipid extract (YPLE), at the polymer's MIC against C. albicans (3 μg/mL). At this and higher concentrations, leakage requires a lag time but then proceeds to 100%. Concerted activity tests imply that the activity of the polymer does not involve detergent-like effects. Both vesicle leakage and antimicrobial activity against C. albicans spheroplasts are independent of the presence of a detergent, octyl glucoside (OG). Negligible activity is found against zwitterionic vesicles or red blood cells. All these characteristics provide a consistent, detailed picture of membrane leakage induced by electrostatic lipid clustering. The cationic/hydrophobic binary copolymer 40:60 MM:CO shows a fundamentally different pattern. Vesicle leakage is transient (limited to <100%) and graded, unspecific between zwitterionic and YPLE vesicles, additive with detergent action, and correlates poorly with biological activity. This activity profile suggests action by membrane asymmetry stress. We conclude that comprehensive leakage experiments can identify the mode of action for model membrane disruption, and we hypothesize that the correlation between vesicle leakage and antimicrobial activity is good for some types of membrane leakage but not for others.

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Virus-Mimicking Polymer Molecular Brushes Are Potent Antibiotics with Double Selectivity

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Evolution of antibiotics-resisting pathogens has become one of the greatest challenges in the battle of bacterial infection. Inspired by the structures of bacteria-invading viruses and antimicrobial peptides, we hypothesize that in addition to a balance of amphiphilicity and electropositivity, the nanoscale architecture is an essential determinant that dictates how membrane-active antibiotics remodel host membranes to achieve desirable activity and selectivity. Here we study the structure-activity relationship of a series of polymer molecular brushes (PMBs) with well-defined nanoscale architectures that mimic spherical and rod-shaped viruses. Our preliminary data based on PMBs with hydrophilic polymer brushes reveal that: (1) amphiphilicity is not a required trait - hydrophilic PMBs can be designed to have potent antibiotic performance as well with no hemolytic side effect; (2) the nanoscale architecture of PMBs defines their double selectivity, not molecular weight per se; (3) PMBs are far more powerful antibiotics than individual linear-chain polymers that make up the PMBs; and (4) nanostructured PMBs induce topological changes of membranes by forming membrane pores that unlikely fit in with any known models of AMP action. These findings challenge existing wisdom and suggest that the spatially-defined, multivalent interactions inherent to PMBs is of great significance for the development of polymer-based antibiotics.

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Analysis of Piscidin and Lipopolysaccharide Interactions: A Step Towards Characterizing Immunomodulation

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Lipopolysaccharide (LPS) is one of the most extensively studied pathogen-associated molecular patterns (PAMPs), as it composes 90% of the membrane in Gram-negative bacteria. Recognition of micellar aggregates of LPS by the immune system subsequently mounts an inflammatory response against infection. However, if this process is over exaggerated, high levels of cytokines may become detrimental, leading to organ shutdown and septic shock. Various cationic antimicrobial peptides (AMPs) have been shown to decrease the transcription and release of pro-inflammatory cytokines associated with LPS recognition.

Piscidin is a cationic antimicrobial peptide first isolated in the mast cells of fish. In the presence of a lipid bilayer, it folds into an amphipathic α-helix structure, which facilitates peptide-lipid bilayer binding. Based on the studies of other cationic AMPs, it was hypothesized that piscidin-1 (P1) and piscidin-3 (P3) would fold into their α-helical state and bind strongly to LPS, breaking apart its large aggregates. Using isothermal calorimetry to explore the thermodynamics of binding, it was shown that both peptides bound strongly to LPS through an exothermic reaction. Furthermore, fluorescence-dequenching studies were carried out using Fluorescein isothiocyanate labeled LPS (FITC-LPS), demonstrating piscidin's ability to separate FITC-LPS aggregates. Finally, both peptides were induced by LPS to be approximately 97% helical from their native random coil state, as shown by circular dichroism. Based upon these results, it is clear that significant interactions occur between LPS and piscidin, warranting future exploration of the effect of piscidin on LPS recognition by immune cells.

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Studying the Mechanisms of Hybrid Peptides Containing Permeabalizing and Cell Penetrating Domains

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Antibiotic resistant pathogens cause 2 million infections and 23,000 deaths in the U.S. every year. Bacteria's adaptability to the current drugs on the market necessitates alternative therapeutics, such as antimicrobial peptides (AMPs). AMPs exhibit non-specific bactericidal activity against both gram positive and negative bacteria, selectively killing prokaryotes over eukaryotes. These peptides function by two main mechanistic categories: 1) permeabilization, where the peptide compromises the membrane causing intercellular leakage, and 2) translocation, where the integrity of the membrane is maintained while the peptide enters the cell and disrupts intercellular processes. Our current study examines the effect of combining AMPs that utilize different antimicrobial mechanisms together to form a hybrid peptide. Based on previous work in our lab considering the mechanism of hipposin, we predicted that hybrid peptides made from combining permeabilizing and translocating peptides would generally follow a permeabilizing mechanism. In particular, we considered the activity of hybrid peptides made from parasin, a previously characterized AMP known to work via permeabilization, and DesHDAP1, a designed peptide known to translocate through bacterial membranes. We attached DesHDAP1 to both the N- and C-termini of Parasin, both with and without an alanine linker between the peptides. The activity and mechanism of these hybrid peptides was considered using radial diffusion assays, propidium iodide uptake assays, and confocal microscopy. Confocal microscopy integrated the formation of larger bacterial spheroplasts and the utilization of a membrane specific dye to test for peptide-membrane co-localization. These enhancements have improved image quality and aid in reliable interpretations of translocation versus membrane localization. Overall, the hybrid peptides did demonstrate significant permeabilizing activity and were at least as active as the parent peptides.